

What is claimed is:

1. A method of determining the time course of a biomolecular reaction comprising

- 50 (a) forming a first reagent mixture containing a reactant, a luminophore and a biomolecular reaction partner wherein the reactant reacts with the biomolecular reaction partner, and the luminophore participates with the
- 55 the biomolecular reaction partner, or a reaction product of the biomolecular reaction partner, to emit electrochemiluminescence upon exposure of the reagent mixture to electrical energy;
- 60 (b) exposing the first reagent mixture to a series of electrical pulses at a preselected potential and at preselected intervals of time and duration, and measuring the electrochemiluminescence at the preselected intervals of time to obtain a value for each interval;
- 65 (c) forming a second reagent mixture having the components contained in the first reagent mixture;
- (d) allowing the second reagent mixture to react until the reaction is complete and then exposing the mixture to

a series of electrical pulses at the preselected potential, intervals of time and duration as performed in step (b) and measuring the electrochemiluminescence at the preselected intervals of time as performed in step (b) to obtain a value for each interval;

- (e) forming a third reagent mixture having the components contained in the first reagent mixture except that it does not contain the biomolecular reaction partner;
- (f) exposing the third reagent mixture to a series of electrical pulses at the preselected potential, intervals of time and duration as performed in step (b) and measuring the electrochemiluminescence at the preselected intervals of time as performed in step (b) to obtain a value for each interval;
- (g) subtracting the value obtained for the first interval in step (f) from the value obtained for the first interval in step (b) to obtain a first difference;
- (h) subtracting the value obtained for the first interval in step (f) from the value obtained for the first interval in step (d) to obtain a second difference;
- (i) dividing the first difference by the second difference to obtain a normalized value for the first interval;
- (j) repeating steps (g), (h) and (i) for each successive interval to obtain a normalized value for each successive interval;
- (k) and determining the time course of the biomolecular reaction from the normalized value of all of the intervals.

2. The method of claim 1 wherein the reactant and the luminophore are combined in a chemical moiety having the formula



wherein M is ruthenium or osmium; P is a polydentate ligand of M; L¹, L², L³, L⁴, L⁵ and L⁶ are ligands of M, each of which may be the same as or different from each other ligand; D is a substance covalently bound to one or more of P, L¹, L², L³, L⁴, L⁵ or L⁶ through one or more amide or amine linkages; m is an integer equal to or greater than 1; each of n, o, p, q, r and s is zero or an integer; t is an integer equal to or greater than 1; u is an integer equal to or greater than 1; and P, L¹, L², L³, L⁴, L⁵, L⁶ and D are of such composition and number that the chemical moiety can be induced to emit electromagnetic radiation and the total number of bonds to M provided by the ligands of M equals the coordination number of M.

3. The method of claim 1 wherein the luminophore is selected from the group consisting of fluorescent or phosphorescent polyaromatic hydrocarbons and fluorescent or phosphorescent transition metal chelates.

4. The method of claim 3 wherein the transition metal chelates are organometallic compounds.

5. The method of claim 1 wherein the luminophore is selected from the group consisting of Ru-containing and Os-containing compounds.

6. The method of claim 1 wherein the luminophore is ruthenium tris-bipyridine or osmium tris-bipyridine.

7. The method of claim 1 wherein the biomolecular reaction is an enzymatic reaction, the reagent mixture contains an enzyme and the reactant is a substrate on which the enzyme exerts catalytic action, and the biomolecular reaction partner is a cofactor.

8. The method of claim 7 wherein the luminophore is selected from the group consisting of fluorescent or phos-

phorescent polyaromatic hydrocarbons and fluorescent or phosphorescent transition metal chelates.

9. The method of claim 7 wherein the enzyme is an oxido reductase.

10. The method of claim 9 wherein the oxido reductase is a dehydrogenase.

11. The method of claim 7 wherein the cofactor is a metal ion.

12. The method of claim 7 wherein the cofactor is a coenzyme.

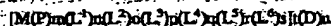
13. The method of claim 12 wherein the coenzyme is in its oxidized form.

14. The method of claim 1 wherein the biomolecular reaction is a binding reaction.

15. The method of claim 14 wherein the binding reaction is selected from the group consisting of antibody-antigen, ligand-receptor, avidin-biotin, base pairing, lectin-carbohydrate, and enzyme-inhibitor.

16. The method of claim 14 wherein the luminophore is selected from the group consisting of fluorescent or phosphorescent polyaromatic hydrocarbons and fluorescent or phosphorescent transition metal chelates.

17. The method of claim 14 wherein the reactant and the luminophore are combined in a chemical moiety having the formula



wherein M is ruthenium or osmium; P is a polydentate ligand of M; L¹, L², L³, L⁴, L⁵ and L⁶ are ligands of M each of which may be the same as or different from each other ligand; D is a substance covalently bound to one or more of P, L¹, L², L³, L⁴, L⁵ or L⁶ through one or more amide or amine linkages; m is an integer equal to or greater than 1; each of n, o, p, q, r and s is zero or an integer; t is an integer equal to or greater than 1; u is an integer equal to or greater than 1; and P, L¹, L², L³, L⁴, L⁵, L⁶ and D are of such composition and number that the chemical moiety can be induced to emit electromagnetic radiation and the total number of bonds to M provided by the ligands of M equals the coordination number of M.

18. A method of determining the time course of an enzymatic reaction comprising

- (a) forming a first reagent mixture containing an enzyme, a reactant on which the enzyme exerts catalytic action, a luminophore and a reaction partner which is a cofactor wherein the reactant reacts with the reaction partner, and the luminophore participates with the reaction partner, or a reaction product of the reaction partner, to emit electrochemiluminescence upon exposure of the reagent mixture to electrical energy;
- (b) exposing the first reagent mixture to a series of electrical pulses at a preselected potential and at preselected intervals of time and duration, and measuring the electrochemiluminescence at the preselected intervals of time to obtain a value for each interval;
- (c) forming a second reagent mixture having the components contained in the first reagent mixture;
- (d) allowing the second reagent mixture to react until the reaction is complete and then exposing the mixture to a series of electrical pulses at the preselected potential, intervals of time and duration as performed in step (b) and measuring the electrochemiluminescence at the preselected intervals of time as performed in step (b) to obtain a value for each interval;
- (e) forming a third reagent mixture having the components contained in the first reagent mixture except that it does not contain the reaction partner;

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- (f) exposing the third reagent mixture to a series of electrical pulses at the preselected potential, intervals of time and duration as performed in step (b) and measuring the electrochemiluminescence at the preselected intervals of time as performed in step (b) to obtain a value for each interval;
- (g) subtracting the value obtained for the first interval in step (f) from the value obtained for the first interval in step (b) to obtain a first difference;
- (h) subtracting the value obtained for the first interval in step (f) from the value obtained for the first interval in step (d) to obtain a second difference;
- (i) dividing the first difference by the second difference to obtain a normalized value for the first interval;
- (j) repeating steps (g), (h) and (i) for each successive interval to obtain a normalized value for each successive interval;
- (k) and determining the time course of the enzymatic reaction from the normalized value of all of the intervals.

19. The method of claim 18 wherein the luminophore is selected from the group consisting of fluorescent or phosphorescent polyaromatic hydrocarbons and fluorescent or phosphorescent transition metal chelates.

20. The method of claim 18 wherein the enzyme is an oxido reductase.

21. A method of determining the time course of a binding reaction comprising

- (a) forming a first reagent mixture containing a reactant, a reaction partner and a luminophore, wherein the reactant reacts with the reaction partner in a reaction selected from the group consisting of antibody-antigen, ligand-receptor, avidin-biotin, base pairing, lectin-carbohydrate, and enzyme-inhibitor, and the luminophore participates with the reaction partner to emit electrochemiluminescence upon exposure of the reagent mixture to electrical energy;
- (b) exposing the first reagent mixture to a series of electrical pulses at a preselected potential and at preselected intervals of time and duration, and measuring the electrochemiluminescence at the preselected intervals of time to obtain a value for each interval;
- (c) forming a second reagent mixture having the components contained in the first reagent mixture;
- (d) allowing the second reagent mixture to react until the reaction is complete and then exposing the mixture to a series of electrical pulses at the preselected potential, intervals of time and duration as performed in step (b) and measuring the electrochemiluminescence at the preselected intervals of time as performed in step (b) to obtain a value for each interval;
- (e) forming a third reagent mixture having the components contained in the first reagent mixture except that it does not contain the reaction partner;
- (f) exposing the third reagent mixture to a series of electrical pulses at the preselected potential, intervals of time and duration as performed in step (b) and measuring the electrochemiluminescence at the preselected intervals of time as performed in step (b) to obtain a value for each interval;
- (g) subtracting the value obtained for the first interval in step (f) from the value obtained for the first interval in step (b) to obtain a first difference;
- (h) subtracting the value obtained for the first interval in step (f) from the value obtained for the first interval in step (d) to obtain a second difference;

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- (i) dividing the first difference by the second difference to obtain a normalized value for the first interval;

- (j) repeating steps (g), (h) and (i) for each successive interval to obtain a normalized value for each successive interval;

- (k) and determining the time course of the binding reaction from the normalized value of all of the intervals.

22. The method of claim 21 wherein the reactant is attached to the luminophore to form a chemical moiety having the formula



wherein M is ruthenium or osmium; P is a polydentate ligand of M; L¹, L², L³, L⁴, L⁵ and L⁶ are ligands of M, each of which may be the same as or different from each other ligand; D is a substance covalently bound to one or more of P, L¹, L², L³, L⁴, L⁵, L⁶ through one or more amide or amine linkages; m is an integer equal to or greater than 1; each of n, o, p, q, r and s is zero or an integer; t is an integer equal to or greater than 1; u is an integer equal to or greater than 1; and P, L¹, L², L³, L⁴, L⁵, L⁶ and D are of such composition and number that the chemical moiety can be induced to emit electromagnetic radiation and the total number of bonds to M provided by the ligands of M equals the coordination number of M.

23. The method of claim 21 wherein the luminophore is selected from the group consisting of fluorescent or phosphorescent polyaromatic hydrocarbons and fluorescent or phosphorescent transition metal chelates.

24. A system for determining the time course of a biomolecular reaction comprising

a first reagent mixture containing as reagents a reactant, a luminophore and a reaction partner wherein the reactant reacts with the reaction partner, and the luminophore participates with the reaction partner, or the reaction product of the reaction partner, to emit electrochemiluminescence upon exposure of the reagent mixture to electrical energy; a second reagent mixture having the components contained in the first reagent mixture except that it comprises reacted reagents; and a third reagent mixture having the components contained in the first reagent mixture except that it does not contain the reaction partner;

a means for separately exposing each of the first, second and third reagent mixtures to a series of electrical pulses at a preselected potential and at preselected intervals of time and duration; and a means for measuring the electrochemiluminescence at the preselected intervals of time.

25. The system of claim 24 wherein the reactant and the luminophore comprise a chemical moiety having the formula



wherein M is ruthenium or osmium; P is a polydentate ligand of M; L¹, L², L³, L⁴, L⁵ and L⁶ are ligands of M, each of which may be the same as or different from each other ligand; D is a substance covalently bound to one or more of P, L¹, L², L³, L⁴, L⁵ or L⁶ through one or more amide or amine linkages; m is an integer equal to or greater than 1; each of n, o, p, q, r and s is zero or an integer; t is an integer equal to or greater than 1; u is an integer equal to or greater than 1; and P, L¹, L², L³, L⁴, L⁵, L⁶ and D are of such

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composition and number that the chemical moiety can be induced to emit electromagnetic radiation and the total number of bonds to M provided by the ligands of M equals the coordination number of M.

26. The system of claim 24 wherein the luminophore is selected from the group consisting of fluorescent or phosphorescent polyaromatic hydrocarbons and fluorescent or phosphorescent transition metal chelates.

27. The system of claim 26 wherein the transition metal chelates are organometallic compounds.

28. The system of claim 24 wherein the luminophore is selected from the group consisting of Ru-containing and Os-containing compounds.

29. The system of claim 24 wherein the luminophore is ruthenium tris-bipyridine or osmium tris-bipyridine.

30. The system of claim 24 wherein the biomolecular reaction is an enzymatic reaction, the reagent mixture contains an enzyme and the reactant is a substrate on which the

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enzyme exerts catalytic action, and the reaction partner is a cofactor.

31. The system of claim 30 wherein the luminophore is selected from the group consisting of fluorescent or phosphorescent polyaromatic hydrocarbons and fluorescent or phosphorescent transition metal chelates.

32. The system of claim 31 wherein the enzyme is an oxide reductase.

33. The system of claim 32 wherein the oxide reductase is a dehydrogenase.

34. The system of claim 31 wherein the cofactor is a metal ion.

35. The system of claim 31 wherein the cofactor is a coenzyme.

36. The system of claim 35 wherein the coenzyme is in its oxidized form.

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